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DRUG DISCOVERY

Quantitative determination of microelements in *Ginkgo biloba* L. leaves, harvested in Ukraine

Lysiuk RM☼, Darmohray RY, Tsal OY, Bitlian VI

***Ginkgo biloba*, that is widely used to treat various diseases due to the numerous bioactive compounds, attracts considerable investigation interest. Trace elements content in *Ginkgo biloba* leaves, a promising nephroprotective flavonoid containing medicinal plant material, collected in two cities of Ukraine, were quantitatively determined by atomic absorption spectrometry with electrothermal atomization (AAS / EA) with a Zeeman background correction and compared with similar currently known studies results from different areas. The analyzed samples of *Ginkgo biloba* leaves seem to be a valuable and non-toxic source of Mn, Cu, Zn and Se and might be recommended for a wider application also as a food supplement.**

INTRODUCTION

The worldwide sales of ginkgo (*Ginkgo biloba, Ginkgoaceae*) leaf products are difficult to estimate but believed to be worth around half a billion USD or more, and establishing plantations to cultivate the species to meet the industrial demand for ginkgo leaf raw material is a promising approach for conserving the species. In many countries such as France, the USA, and China, plantations have been established to cultivate *G. biloba* for the supply ofginkgo leaves to the pharmaceutical industry [8].

The maidenhair tree is the only survivor of an order widely represented until the end of the tertiary era. The trees can live for hundreds of years. The tree flowers for the first time when it is between 20 and 30 years old [5].Ginkgo is indigenous to China, Japan and Korea, and is also found in Europe and the U.S. [6].

Folium Ginkgo contains a wide variety of phytochemical, including alkanes, lipids, sterols, benzenoids, carotenoids, phenylpropanoids, carbohydrates, flavonoids, and terpenoids. The major constituents are flavonoids of which mono-, di-, and tri-glycosides and coumaric acid esters that are based on the flavonols kaempferol and quercetin dominate [6]. Lesser quantities of glycosides are derived from isorhamnetin, myricetin, and 3- methylmyricetin. Nonglycosidic biflavonoids, catechins, and proanthocyanidins are also present. Characteristic constituents of this plant material are the unique diterpene lactones ginkgolides A, B, Cand J, and the sesquiterpene lactone bilobalide [21].

The plant material *Folium Ginkgo*is included into numerous Pharmacopoeias. There are the following requirements for contents of active principles: not less than 0.5 per cent of flavonoids, calculated as flavone glycosides [3]. The United States Pharmacopeia, besides of flavonol glycosides (not less than 0.5%), additionally standardizes the terpene lactones content: not less than 0.1%, calculated as the sum of bilobalide, gingkolide A, gingkolide B, and gingkolide C [20].

Department of Pharmacognosy and botany, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine;
☼*Corresponding Author:* Lysiuk R.M.; Email: pharmacognosy.org.ua@ukr.net

Standardized ginkgo leaf extracts are among the herbal preparations that have undergone most extensive clinical investigation. The effects of ginkgo extracts in dementia have been tested clinically mostly in trials involving patients with cognitive deficiency, Alzheimer's disease and/or multi-infarct dementia [2].

In clinical studies *G. biloba* effectively managed symptoms of cerebral insufficiency including difficulty in concentration and memory, absent-mindedness, confusion, lack of energy, tiredness, decreased physical performance, depressive mood, anxiety, dizziness, and headache. Several mechanisms of action of the plant have been described: effects on blood circulation such as the vasoregulating activity of arteries, capillaries, veins (increased blood flow), rheological effects, metabolic changes such as increased tolerance to anoxia, beneficial influence on neurotransmitter disturbances and prevention of damage to membranes by free radicals [21].

Continuing the research of trace elements in promising nephroprotective flavonoid containing herbal substances [1, 10, 14], the gingko leaf was selected as an object for analysis, considering that data units microelements contents are rather limited and do not occur publications concerning research outcomes on the samples, collected in Europe, as well in the Western and Southern parts of Ukraine (Lviv and Odessa).

MATERIALS AND METHODS

The plant materials, consisting of the dried leavesof *Gingko biloba* were collected in June, 2017 in the cities of Lviv and Odessa. Sample 1 - *Gingko biloba* leaf, collected from female tree at the Botanical garden of Danylo Halytsky Lviv National Medical University (LNMU). Sample 2 – *Gingko biloba* leaf, harvested in the city of Odessa. Sample 3 - *Gingko biloba* leaf, collected from male tree at the Botanical garden of Danylo Halytsky LNMU.

Microelements content in leaves of *Gingko biloba* were quantitatively determined by atomic absorption spectrometry with electrothermal atomization (AAS / EA) with a Zeeman background

Table 1
Parameters of atomic absorption spectrometry for analysis of trace elements As, Co and Zn in the plant material

Trace element	Wavelength of absorption (resonance) lines, nm	Voltage of resonance radiation lamp, mA	Flow rate of argon, L / min	Ashing temperature, ° C	Atomization temperature, ° C	Slit width, nm
As	193,7	10	0,3 .	1400	2600	0,5
Co	242,5	10	0,3 .	750	2300	0,2
Zn	307,6	7	0,3 .	300	1900	1,0

Table 2
Contents of trace elements in the analyzed samples of *Gingko biloba* leaves, collected in Ukraine

Gingko leaf, sample	Pb µg/g	Cd µg/g	As µg/g	Mn µg/g	Se µg/g	Cu µg/g	Co µg/g	Zn µg/g
1	0.79	0.007	0.03	7.15	0.43	2.27	0.037	5.7
2	0.46	0.018	0.04	15.2	0.41	2.7	0.07	7.9
3	0.77	0.007	0.04	5.5	0.40	1.8	0.031	2.9

correction after total microwave – assisted digestion of the plant material samples by means of their conversion into soluble forms and further determination for concentration in the solutions (an official method of standard additions) [3] in sealed analytical autoclaves, in accordance with the technique, described in [14].

The current study of the contents of microelements have been carried out by AAC/EA method on VARIAN AA 240Z after mineralization in the microwave Milestone Start D.

Data related to applied reagents, preparation of the samples (from 0,6g), blank and reference solutions, also as parameters of temperature changes over time of mineralization in autoclaves, and of AAS for analysis of 5 trace elements in the subjected plant materials are presented in our earlier publication [14]. Parameters of AAS / EA for analysis of As, Co and Zn are shown in the table 1.

To construct a calibration graph were used reference solutions of different concentrations of the trace elements (within the operating range for determination of the spectrophotometer) obtained from the blank solutions (0.1 g/cm^3) by the standard addition method [3].

RESULTS & DISCUSSION

Trace elements in medicinal plants can have a positive contribution as a source of essential nutrients or even as active principles, or a negative effect caused by the accumulation of high concentrations of potentially toxic compounds [17]. Mineral concentrations in herbs, used for renal and urinary tract disorders, have been reported to be higher than in non-medicinal plants [18]. Chronic kidney disease is associated with low concentration of serum selenium and lower platelet glutathione peroxidase activity [11]. Zinc deficiency has been suggested as a possible cause of some typical uremic symptoms [16]. Serum concentrations of manganese, that is a part of enzymes involved in urea formation, were found to be low in chronic hemodialysis patients [7].

Gingko leaf extract deserves a particular attention as a nephroprotective agent, since it ameliorates gentamicin-induced nephrotoxicity in rats, significantly preventing changes in blood urea, serum creatinine and creatinine clearance [15] and the extract has been used widely in China as a supplement to improve albuminuria and kidney function during the early stage of diabetic nephropathy [23].

The found quantities (expressed in µg/g) of eight trace elements in the analyzed samples of *Gingko biloba* leaves, harvested in the cities of Lviv and Odessa, are presented in the Table 2.

The received quantities of eight analyzed trace elements in *Gingko* leaves allow their division into three groups: Mn, Cu and Zn (the content ranges from 1,8 to 15,2 µg/g), Se and Pb (0.40 - 0.79 µg/g), Cd, As and Co (0,007-0,07 µg/g).

The determined amounts of lead and cadmium in all investigated samples of leaves of *Gingko biloba* comply with the requirements of the European Pharmacopoeia (Monograph 01/2012:1433 “Herbal Drugs): maximum 1.0 ppm of cadmium and maximum 5.0 ppm of lead[3], and therefore, might be considered as non-toxic natural source of the essential microelements manganese, copper and selenium.

Analysis of literature data allowed finding of 6 studies [4, 9, 12, 13, 19, 22], dedicated on quantitative estimation of trace elements in ginkgo leaves, collected from several locations and investigated by various analytical techniques.

Yu *et al.* (1992) [22] have studied the content of trace elements in *Ginkgo* leaves and found 0.1–2.17 ppm of Se, 2.8–6.9 ppm of Cu, 6.1–17.1 ppm of Zn, and 15–73 ppm of Mn.

Herbal tea samples purchased in Nakhon Pathom, Thailand were digested with nitric acid and analyzed for 11 heavy metals by inductively coupled plasma mass spectrometry (ICP-MS). Gingko leaf yielded: As - 0.001, Cd - 0.086, Pb - 7.374, Zn - 32.083, Cu - 7.219, Mn - 32.427mg/kg [13].

Microwave digestion technique was used in the decomposition of *Ginkgo biloba* leaves and the contents of Cu, Zn and Zn/Cu were determined by flame atomic absorption spectrometry to study the distribution rule of metallic elements in the trees at the same age and in the same area. The contents of copper from 1.50 to 3.05 µg/g, while Zn/Cu values were from 2.68 to 5.93 µg/gin the leaves of 6 different trees in the same growing area [9].

The contents of Cu, Zn, Mn was high, especially Se in *Ginkgo biloba* L., determined by graphite furnace atomic absorption spectrometry (GFAAS), [12]. The yield of trace elements Se, Cd and Pb in ginkgo via GFAAS and standard addition method was investigated. In ginko leaf Se contents was 16.91, Cd 0.2020, Pb 4.021µg/g [4].

In the study [19], 15 samples of Chinese therapeutic foods and herbs, that are frequently consumed by people in both the East and West, were analyzed by AAS for the content of trace elements, including cadmium, lead, arsenic, copper and zinc. *Folium Ginkgo*

biloba contained: Cd - 0.07±0.04, Cu - 12.47±2.42, Pb - 1.28±0.24, Zn - 2.37±0.21mg/kg, As was not detected**.**

Therefore, comparing received experimental data and research outcomes of 6 aforementioned studies for quantitative estimation of trace elements in ginkgo leaves, harvested in different areas, might be made the following conclusions: samples, collected in Ukraine, contained considerably less amounts of toxic elements Pb and Cd, also as less quantities of Mn, Cu and Se, comparing with the plant material samples from China and Thailand. Co contents could not be compared considering absence of data. The content of arsenic in subjected plant samples were considerably higher than in ones from Thailand [13]. The obtained Zn yield lie in the wide range of amounts, determined for other locations (from 2,37 to 32,083 μg/g).

CONCLUSIONS

Ginkgo biloba is an ancient medicinal tree species that has been in existence for millennia without undergoing modifications due to its resistance to environmental stresses. Medicinal use of the species is attracting research interest, especially the various parts of the tree that are used in orthodox or traditional medicine to treat diseases due to the many bioactive compounds. In many countries, the cultivation of plantations for the supply of ginkgo leaf‑based pharmaceutical formulations is in progress, and efforts to standardize ginkgo leaf extract as herbal medication for human use are being made. Trace elements content in leaves of *Ginkgo biloba*, a promising nephroprotective flavonoid containing herbal substance, collected in two cities of Ukraine (Lviv and Odessa) were quantitatively determined by AAS / EA with a Zeeman background correction after total microwave – assisted digestion of the plant material samples and compared with similar currently known research outcomes from various locations. The subjected to examination samples of *Ginkgo biloba* leaves seem to be a valuable and non-toxic source of microelements manganese, copper, zinc and selenium and might be recommended for a wider application also as a food supplement.

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